Nota: laporan makmal CEPP di mukasurat 11 mengesahkan bahawa TREBEST Plus 300mg tidak mengandungi bahan terlarang.

DETERMINATION OF SILDENAFIL AND TADALAFIL IN TREBEST PLUS CAPSULE FOR ERECTILE DYSFUNCTION BY THIN LAYER CHROMATOGRAPHY (TLC) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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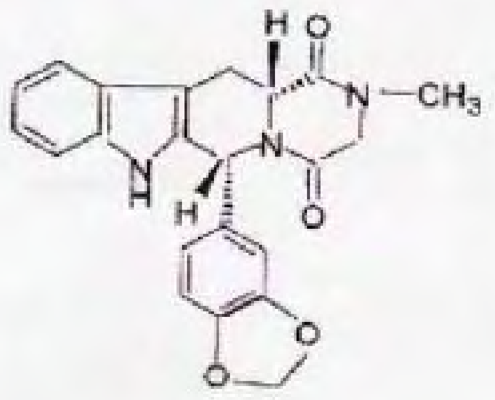
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DETERMINATION OF SILDENAFIL AND TADALAFIL IN TREBEST PLUS CAPSULE FOR ERECTILE DYSFUNCTION BY THIN LAYER CHROMATOGRAPHY (TLC) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

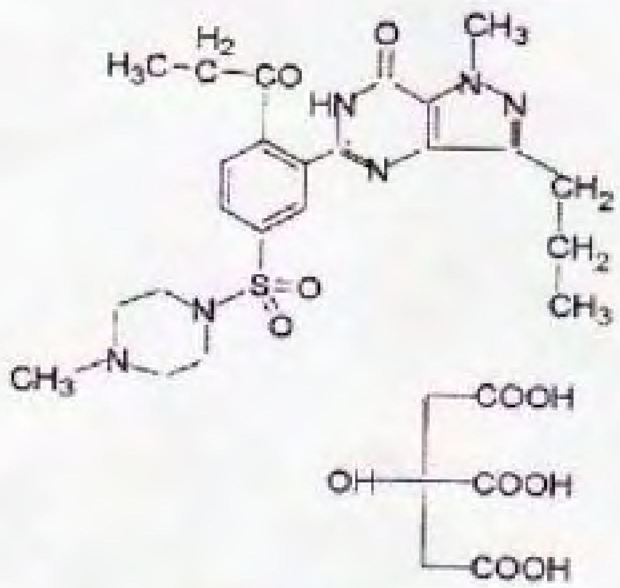
1. INTRODUCTION

Erectile dysfunction (ED), inability to achieve a penile erection sufficient for satisfactory sexual performance, is estimated to affect many men world wide [1], [2] and [3]. ED is more common in advanced age [3], [4] and [5] and related to hypertension or diabetes mellitus or use of certain pharmacological agents e.g. antihypertensives and antihypertensive [1]. ED has been treated by the drugs that inhibit the enzyme phosphodiesterase type 5 (PDE5) activities. Sildenafil [6] was first drug for this purpose and another remarkable achievement in this direction is the introduction of second phosphodiesterase inhibitor called tadalafil, which was launched in February 2003 [7]. Sildenafil is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin- 5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate. Sildenafil is a white to off-white crystalline powder with a solubility of 3.5 mg/ml in water and a molecular weight of 666.7.

Tadalafil is chemically known as pyrazino [1',2':1,6]pyrido[3,4-b]indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)- 2,3,6,7,12,12 a-hexahydro-2-methyl-,(6R-trans)-(6R-,12aR) 2,3,6,7,12,12a-hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl] pyrazino [1',2':1,6] pyrido[3,4-b] indole-1,4-dione (Figure 1). Tadalafil is a selective phosphodiesterase type 5 inhibitor, which is used to treat mild to severe ED in man. Drug testing is an integral part of pharmaceutical analysis and routine quality control monitoring of drug release characteristics. To the best of our knowledge no report has been published on the analysis of tadalafil in pharmaceutical preparations.



Tadalafil



Sildenafil Citrate

Figure 1: Chemical structures of tadalafil and sildenafil.

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2. EXPERIMENTAL PROCEDURES

TREBEST plus capsule
VIAGRA and CIALIS tablets

2.1 Extraction of tadalafil and sildenafil from tablets

To avoid any interference in HPLC separation, the sugar part (outer layer) of the tablet (Viagra and Cialis) were removed by sculpture and the inner part of the tablet was ground to a fine powder. The powder tablets and caplsule was extracted with 5 mL methanol by shaking at 80 °C and then cooled. Methanol was filtered and the residue again was extracted with methanol (5 mL) twice as described above. Methanol extracts were combined together used for chromatographic studies.

2.2 Methodology

A method has been developed for the determination of sildenafil and tadalafil compound in the medicines for erectile dysfunction. The methods that are using are *Thin Layer Chromatography* (TLC) and *High Performance Liquid Chromatography* (HPLC) for for qualitative analysis. Pictures for the extracted materials (Figure 2) and TLC profile (Figure 3) are as below:



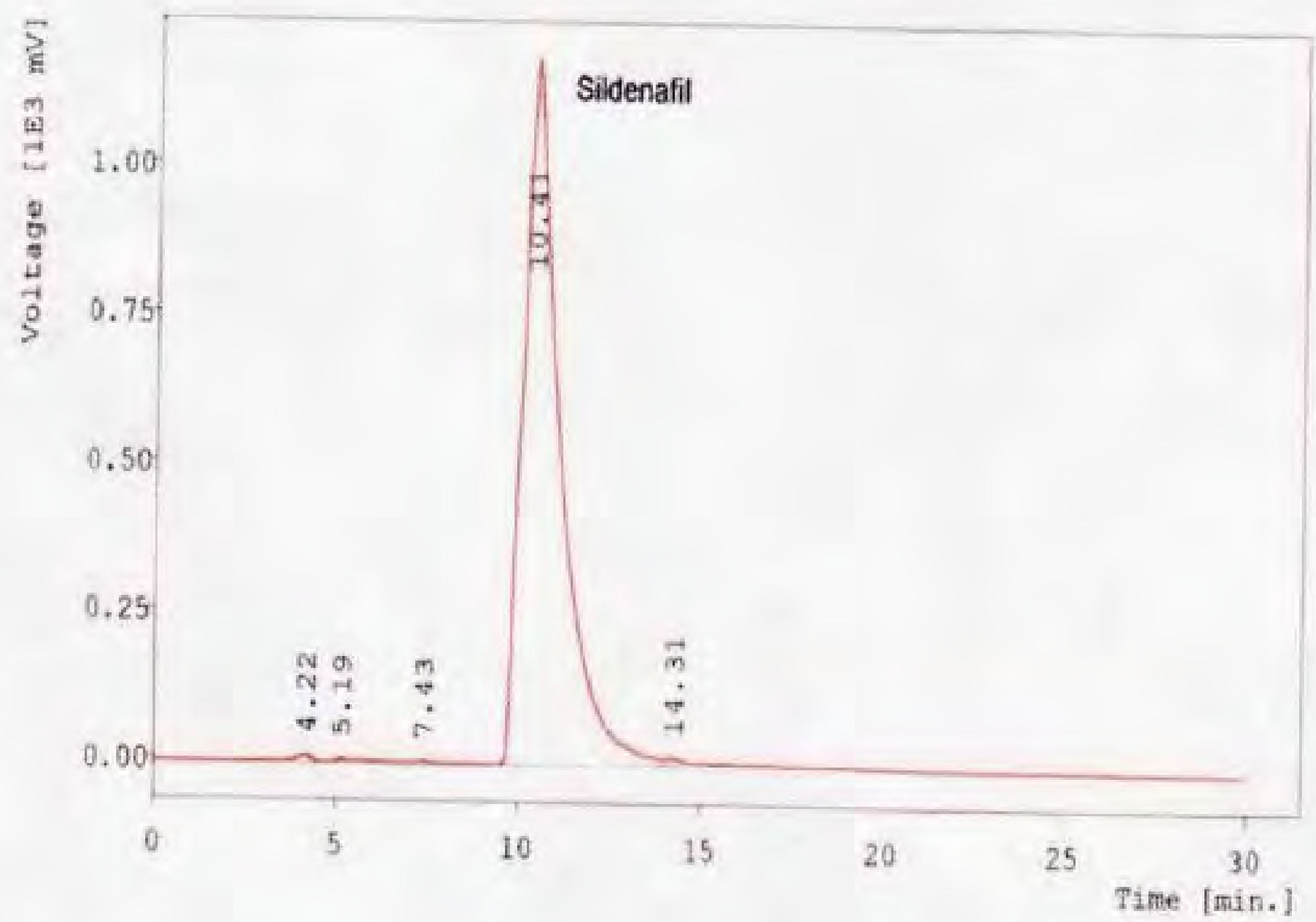
Figure 2: TREBEST plus extracted (yellow) with other medicines in the market

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Figure 3: TLC profile of Trebes plus (T) compared with the Viagra (V) and Cialis (C)

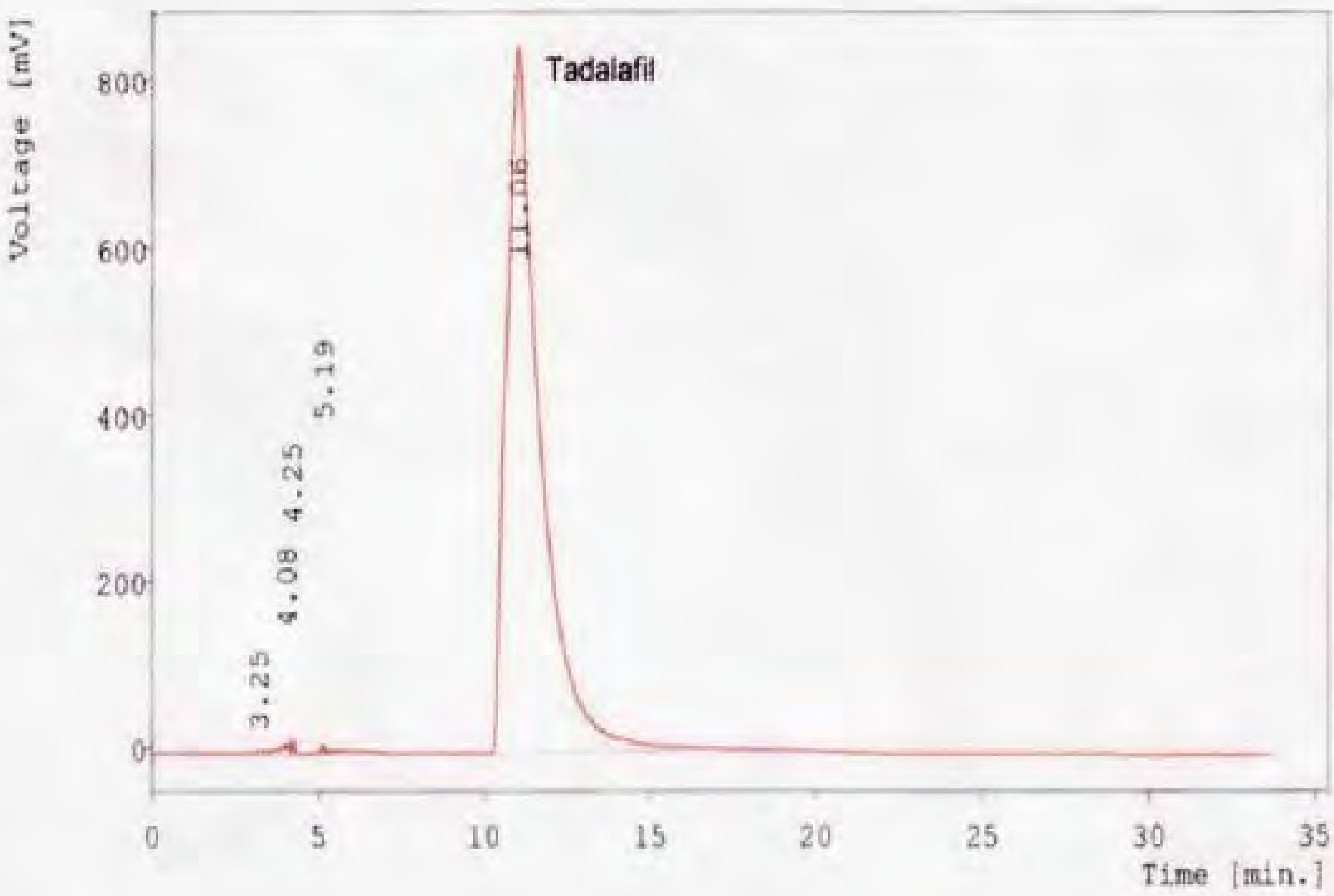
3. RESULTS AND DISCUSSIONS



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	w05 [min.]	Area [%]	Height [%]
1	4.220	327.0910	11.439	0.333	0.385	0.933
2	5.193	348.9127	7.518	0.173	0.411	0.613
3	7.433	116.4923	3.576	0.300	0.137	0.292
4	10.413	83531.8789	1190.370	1.007	98.422	97.121
5	14.307	546.8798	12.758	0.493	0.645	1.041
-	Total	84871.2547	1225.662			

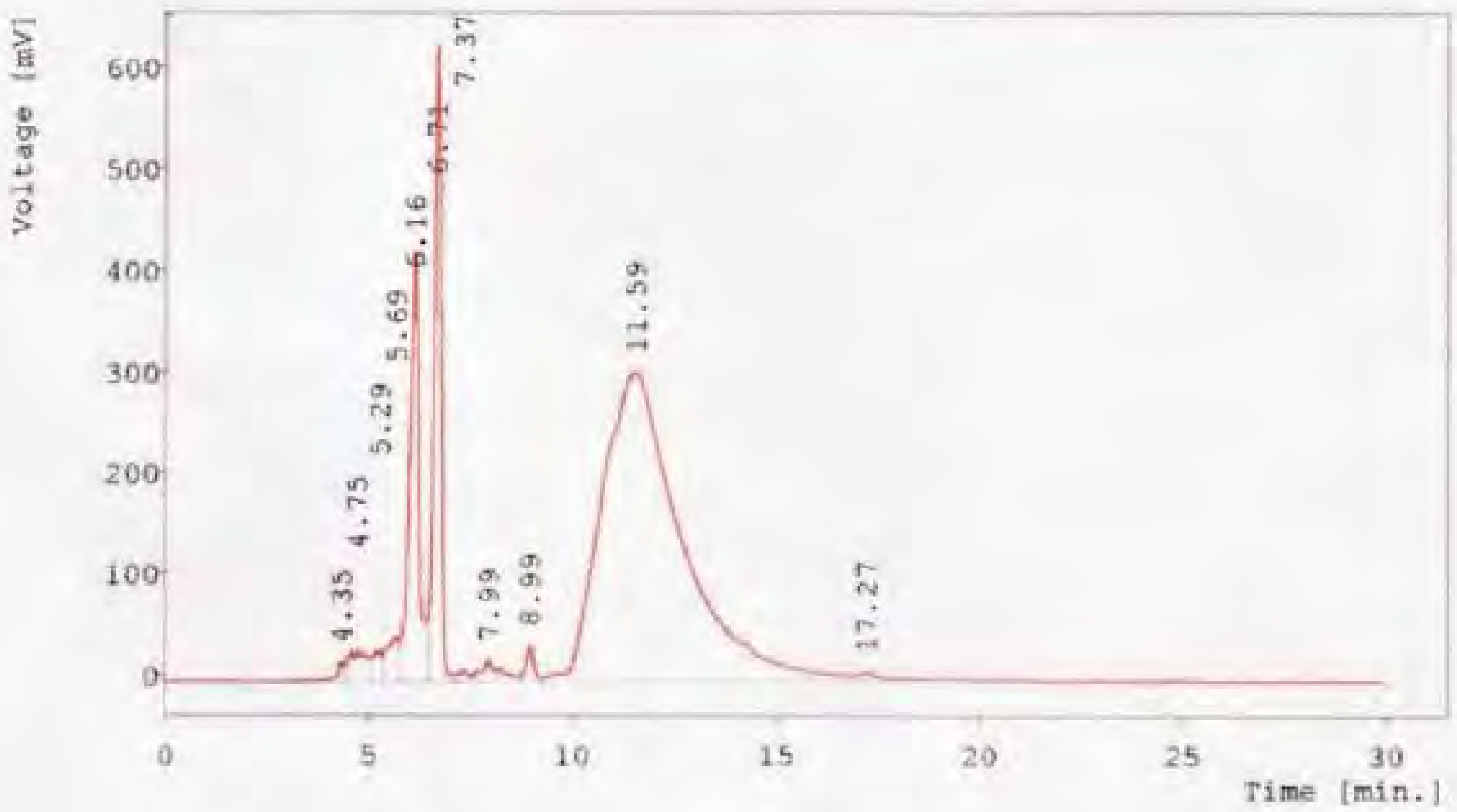
Figure 4: Calibration graph for Viagra (sildenafil)



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	w05 [min.]	Area [%]	Height [%]
1	3.253	58.1334	3.117	0.360	0.097	0.350
2	4.080	233.3434	11.583	0.327	0.389	1.301
3	4.247	106.6358	18.370	0.093	0.178	2.064
4	5.193	104.9460	9.079	0.127	0.175	1.020
5	11.060	59464.5557	847.996	1.007	99.161	95.265
-	Total	59967.6143	890.146			

Figure 5: Calibration graph for Cialis (tadalafil)



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.353	243.5737	20.259	0.120	0.399	1.298
2	4.753	945.0030	31.375	0.620	1.546	2.010
3	5.287	483.3406	31.456	0.300	0.791	2.015
4	5.693	865.0727	43.069	0.400	1.416	2.759
5	6.160	7010.4643	424.120	0.240	11.471	27.166
6	6.713	8068.0308	630.263	0.200	13.202	40.370
7	7.367	197.0696	10.464	0.407	0.322	0.670
8	7.987	679.1017	20.833	0.253	1.111	1.334
9	8.993	489.7712	35.245	0.193	0.801	2.258
10	11.587	41766.0677	306.117	1.967	68.341	19.608
11	17.267	366.7586	8.008	0.607	0.600	0.512
-	Total	61114.2541	1561.208			

Figure 6: Calibration graph for TREBEST plus

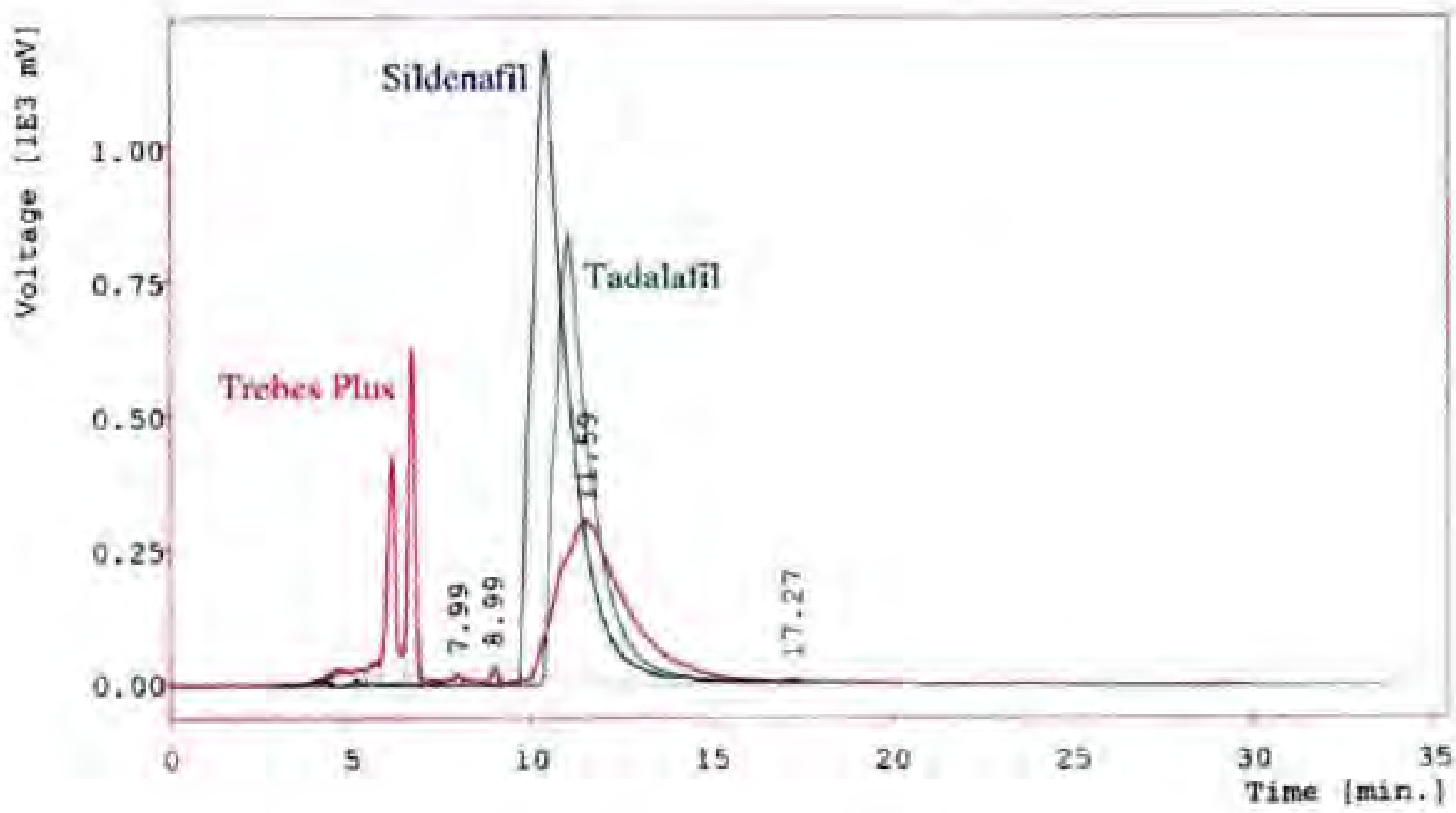


Figure 7: Combination of all calibration graphs

Based on the results obtained, there are no sildenafil and tadalafil in the TREBEST plus capsule. Comparison graph between TREBEST plus capsule, Viagra and cialist tablets, showing absolutely no peak or curve which represents sildenafil and tadalafil compound, indicated that TREBEST plus capsule was free from both sildenefil and tadalafil.

4. CONCLUSION

The developed chromatographic and extraction methods are simple, reliable and reproducible for the analysis of sildenafil and tadalafil in pharmaceutical formulation. The reported method can be used for effective qualitative and quantitative analysis of sildenafil and tadalafil in other pharmaceutical preparations.

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OUR REFERENCE : Lab. Ref./Trebest Biotech/01

28 December, 2005

YOUR REFERENCE :

CERTIFICATE OF ANALYSIS

COMPANY : TREBEST BIOTECH

Dear Sir,

The analysis test results for the sample : **TREBEST Plus Capsule**

1) Thin Layer Chromatography (TLC)

Sildenafil	:	Not Detected
Tadalafil	:	Not Detected

2) High Performance Liquid Chromatography (HPLC)

Sildenafil	:	Not Detected
Tadalafil	:	Not Detected

Analyzed by :

Ms. Harisun Bt. Yaakob
Research Officer,
CEPP, UTM.

Approved by :

Prof. Dr. Mohamad Roji Bin Sarmidi
R&D Manager,
CEPP, UTM.

Thank you.

* The report must not be used for advertising purposes